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absence of polysialic acid-expressing tumor cells in TMA samples was a strong unfavorable prognostic factor for overall survival in advanced disease ($P = 0.0004$), especially when MYCN was not amplified. Polysialic acid-expressing bone marrow metastases were easily detected in smears, aspirates and biopsies. Polysialic acid appears to be a clinically significant molecular marker in neuroblastoma, with potential value in neuroblastoma risk stratification.

Program/Abstract# 74

Remodeling of N-glycosylation pathway of the methylotrophic yeast Hansenula polymorpha: evaluation of the ALG3 deletion strain blocked in the lipid-linked oligosaccharide assembly as a host for the production of therapeutic glycoproteins

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The thermotolearant methylotropic yeast *Hansenula polymorpha* has some advantages over the traditional yeast *Saccharomyces cerevisiae* in the production of recombinant glycoproteins for human therapeutic use, such as less hypermannosylation and lack of highly immunogenic terminal α-1,3-linked mannose residues. As a first step toward humanizing *H. polymorpha*N-glycosylation pathway, we developed the *H. polymorpha och1*Δ mutant strain, having a defect in the outer chain initiation on the core oligosaccharide $Man_8GlcNAc_2$, with the targeted expression of *Aspergillus saitoi*α-1,2-mannosidase in the ER. The engineered *H. polymorpha och1*Δ strain produced the human high mannose-type $Man_5GlcNAc_2$ oligosaccharide as a major *N*-glycan. As an alternative approach, we carried out the remodeling of core oligosaccharide assembly pathway by additional deletion of the*H. polymorpha ALG3* gene, encoding a dolichyl-phosphate-mannose dependent α-1,3-mannosyltransferase. The engineered double deletion (*Hpalg3*Δ*Hpoch1*Δ) strain expressing *A. saitoi*α-1,2-mannosidase generated mainly the trimannosyl-core form glycan ($Man_3GlcNAc_2$), an intermediate for further maturation to human-like complex *N*-glycans.We have performed subsequent modification of *H. polymorpha* glycosylation pathway to synthesize the complex-type *N*-glycans with a terminal *N*-acetyl glucosamine in the glycoengineered Δ*Hpoch1* and*ΔHpoch1*Δ*Hpalg3* strains, respectively. Several combinatorial synthetic leaders were constructed for the localized expression of active human β-1,2*N*-acetyl glucosaminyl transferase I at the Golgi membrane, and the production of complex-type glycans with mono-antennary *N*-acetyl glucosamine was analyzed by a capillary electrophoresis of ATPS-labeled cell wall glycans. The comparative analysis strongly suggested that the Δ*Hpoch1* singledeletion strain would be a more suitable host for further manipulation toward human complex-type *N*-glycansthan the*ΔHpoch1*Δ*Hpalg3* double deletion strain in the aspects of the glycosylation site occupancy and the byproduct $Hex_5GlcNAc_2$ formation.

Program/Abstract# 75

Chemical protein glycosylation: a new approach to protein stabilization

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Protein pharmaceuticals have outstanding potential in the cure and prevention of diseases and have already substantially expanded the field of molecular pharmacology.Unfortunately, proteins (and peptides) frequently display substantial chemical and physical instabilities hampering their success as drugs.Detrimental stresses encountered during manufacturing, storage, delivery, and other pharmaceutically relevant processes, frequently alter the chemical composition and the three-dimensional structure of proteins thus negatively impacting their therapeutic efficacy and giving rise to potential safety hazards for patients (*e.g.*, immune reactions triggered by protein aggregates).This has prompted an intense search for novel strategies to stabilize pharmaceutical proteins.Due to the well known effect of glycans in increasing the overall stability of glycoproteins, rational manipulation of the glycosylation parameters through glycoengineering could become a promising approach to improve both the *in vitro* and *in vivo* stability of protein-based pharmaceuticals.The intent of this presentation is to survey the different physicochemical instabilities displayed by proteins during their pharmaceutical employment, how these can be prevented by glycosylation, and to discuss the currently proposed biophysical models by which glycans induce these stabilization effects.

Program/Abstract# 76

Glycan analysis of a plant-cell derived glucocerebrosidase as a tool for monitoring changes in growth condition and manufacturing

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Glucocerebrosidase (GCD) is a glycoprotein having 4 occupied glycosylation sites. It is incorporated into human macrophage cells via cell surface mannose receptors and catalyzes the hydrolysis of glucosylceramide (glucocerebro-